IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application f: Waldman, S.A. *et al.*

Serial No.: 09/819,252 Group Art Unit: 1642

Filed: March 27, 2001 Examiner: Yu, Misook

Title: Compositions and methods for identifying and targeting cancer cells of alimentary canal origin

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

DECLARATION OF DR. SCOTT A. WALDMAN UNDER 37 CFR 1.132

I, Scott A. Waldman, M.D., Ph.D., do hereby declare:

1. I am the co-inventor of the subject matter claimed in the above-identified patent application.

2. Experiments were performed by me or by others in my laboratory under my supervision to compare the level of expression of Cdx2 in samples of normal esophagus and esophageal cancer samples.

3. Cdx2 mRNA and B-actin mRNA were quantified by quantitative RT-PCR (qRT-PCR) on 17 samples including 4 samples of esophageal cancer and 13 samples of normal esophagus. B-actin mRNA was quantified as a positive control.

4. The data is shown in Tables 1 and 2, attached hereto as Exhibits 1 and 2.

5. The Y axis shows the ratio of Cdx2 expression to B-actin (Cdx2 copy #/B-actin copy #). The individual sample numbers are plotted on the X axis. In Table 1, the esophageal cancer samples assayed are shown as samples 1-4. The 13 normal esophagi assayed are shown as samples 7-19. Lanes 5-6 are blank.

6. The data in Table 2 demonstrate the expression of Cdx2 in the lowest-expressing esophageal tumor (Sample 2 in Table 1, which is Sample 1 in Table 2) compared to that in the 13 normal esophagi samples (Samples 2-14 in Table 2).

7. The data in Tables 1 and 2 demonstrate that Cdx2 is expressed in esophageal cancer but not in normal esophagus. The data in Table 1 show that the ratio of Cdx2 expression to B-actin expression is detectably higher for each cancer sample compared to each normal sample. Table 2 shows that even in the cancer sample with the lowest ratio, the ratio was significantly higher than that in the normal samples. The data in Tables 1 and 2 state that range of expression of Cdx2 (Cdx2 copy #/B-actin copy #) in tumors was 2.50-191.00 whereas in normal esophagus, it was 0.00-0.03. These data support the assertion that Cdx2 is expressed in esophageal cancer samples and not in normal esophagus samples.

8. A copy of the Abstract of Akashi Eda, Hiroyuki Osawa, Kiichi Satoh, Ichiro Yanaka, Ken Kihira, Yumiko Ishino, Hiroyuki Mutoh, Kentaro Sugano, Aberrant expression of CDX2 in Barrett's epithelium and inflammatory esophageal mucosa, Journal of Gastroenterology, Volume 38 Issue 1 (2003) pp 14-22 is attached hereto as Exhibit 3. The data reported therein indicate that Cdx2 is not expressed in normal esophagus.

9. I hereby declare that all statements made herein are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment or both, under section 1001 of Title 18 of the United states Code and that such willful false statements may jeopardize the validity of the applications and any patent issued thereon.

Date:

Scott A. Waldman, M.D. PhD

Exhibit 1: Table 1

Exhibit 2: Table 2

Exhibit 3: Abstract of Akashi Eda, Hiroyuki Osawa, Kiichi Satoh, Ichiro Yanaka, Ken Kihira, Yumiko Ishino, Hiroyuki Mutoh, Kentaro Sugano, Aberrant expression of CDX2 in Barrett's epithelium and inflammatory esophageal mucosa, Journal of Gastroenterology, Volume 38 Issue 1 (2003) pp 14-22

EXHIBIT 1

Table 1

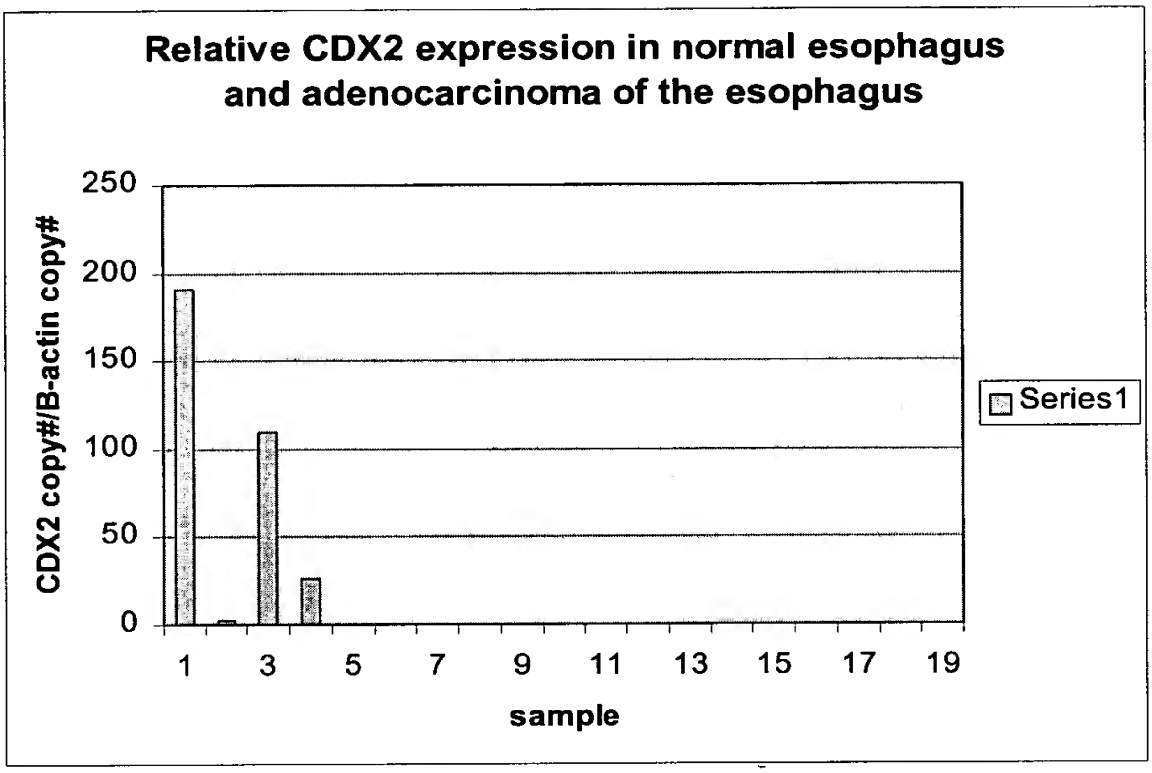

EXHIBIT 2

Table 2

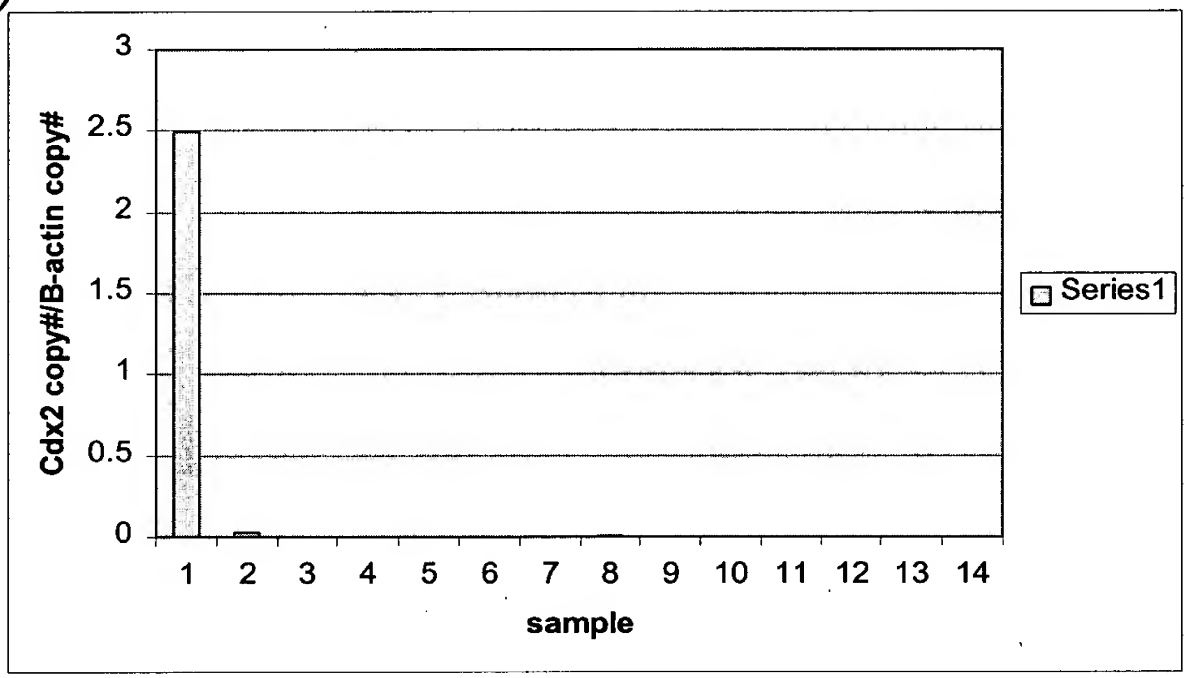

EXHIBIT 3

Gastro-enterology

Aberrant expression of CDX2 in Barrett's epithelium and inflammatory esophageal mucosa.

Eda A, Osawa H, Satoh K, Yanaka I, Kihira K, Ishino Y, Mutoh H, Sugano K.

Division of Gastroenterology, Department of Internal Medicine, Jichi Medical School, Yakushiji, Kawachi, Tochigi 329-0438, Japan.

BACKGROUND: There have been no detailed reports directly comparing the expression of CDX1 with that of CDX2 in the inflammatory esophageal mucosa and Barrett's epithelium. The present study was designed to examine the expression of CDX 1/2 in inflammatory esophageal mucosa with or without Barrett's epithelium. METHODS: The expression of CDX1/2 genes was analyzed using the reverse transcriptase-polymerase chain reaction (RT-PCR) in 34 human esophageal biopsy specimens, and CDX2 expression was also evaluated immunohistochemically, using anti-human CDX2 monoclonal antibody. The biopsy specimens for RNA extraction were taken endoscopically from esophageal mucosa with mucosal break due to gastroesophageal reflux disease (GERD), Barrett's epithelium, and normal epithelium. The expressions of mucin markers (MUC2) and intestine-specific genes (sucrase-isomal-tase, human defensin-5, alkaline phosphatase) were also comparatively analyzed. RESULTS: CDX1/2 expression was not found in the normal esophageal mucosa. The prevalence of CDX1/2 mRNA expression was significantly higher in the mucosa with Barrett's epithelium than in the mucosa without Barrett's epithelium. It is noteworthy, however, that the CDX2 mRNA expression was initiated at the stage of esophagitis, when neither CDX1 nor intestine-specific genes had emerged yet. In contrast to CDX2, CDX1 was expressed only in Barrett's epithelium. Immunohistochemical study demonstrated strong and extensive nuclear immunoreactivity for CDX2 in Barrett's epithelium. Furthermore, fine granular cytoplasmic staining was also observed in the cytoplasm in Barrett's epithelium, as well as in inflammatory esophageal mucosa. CONCLUSIONS: We report here, for the first time, that CDX2 is expressed in patients with Barrett's epithelium and inflammatory esophageal mucosa. These findings imply that the expression of CDX2 may be an early event leading to the development of Barrett's esophagus.